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ORIGINAL PAPER

EVALUATION OF MINERALS, PHENOLICS, RADICAL SCAVENGING ACTIVITY, TOTAL OXIDANT STATUS AND TOTAL ANTIOXIDANT STATUS OF *NEPETA VISCIDA* BOISS.

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Abstract

In this study, some minerals and total phenolic content, radical scavenging activity, total oxidant status, and total antioxidant status of endemic *Nepeta viscida* species were investigated. Total phenolic content of *Nepeta viscida* methanol and acetone extracts was determined as gallic acid equivalent. In the extracts, these values were determined as 128.86 ± 4.52 and 134.78 ± 9.3 µg GAE / mg extract, respectively. Butylated hydroxytoluene (BHT) was used as a standard for radical scavenging effect. It was observed that the radical scavenging effect of *Nepeta viscida* methanol extract was similar to that of the synthetic antioxidant BHT and higher than the effect of acetone extract. Total antioxidant status (TAS) , Total oxidant status (TOS) were measured and OSI values were calculated. Mineral substances included in the species were identified by ICP-OES. *Nepeta viscida* contained elements (Mn, Zn, Se, Fe, Cu) that were incorporated in the antioxidant enzyme structure. Furthermore, it was considered that total phenolic content found in *Nepeta viscida* could be used in phytotherapeutic studies, especially in antioxidative effect studies due to their impact against radicals.

Key words: *Nepeta viscida*, radical scavenging activity, TAS, TOS, total phenolic content.

INTRODUCTION

Lamicaceae family includes a group of annual or perennial herbaceous plants, bush, and trees. The plants in this family that include mint, thyme, and lavender are named as ornamental plants and spices. Volatile and aromatic oils are found in the seborrheic glands in the leaves (Alim et al. 2009; Başer and Kırımer, 2006; Dinesh et al. 2010)

The genus Nepeta of the Lamicaceae family, grows at 0-4500 m, especially at an altitude of 1000-3000 m, in different habitats. It is a blooming plant and includes about 280 species that grow in the southern Europe and in the central and southern parts of Asia. In Turkey, the existence of 44 Nepeta species, 22 of which are endemic, was determined. Previous studies reported that certain Nepata species had antioxidant, booster effects, treats colds, and effective against atherosclerosis since it decreases lipid and protein levels (Adiguzel et al., 2009; Aydın et al. 1999; Bourrel et al., 1993).

Endemic *Nepeta viscida* is a species with no close family members. It is a perennial species growing up to 35-60 cm tall. *Nepeta viscida* has light purple flowers and grows on 740-1800 m high rocky slopes. When assessed with the criteria, due to the possibility of future candidate for extinction definition, it was classified as Near Threatened (Martin et al., 2013; Takedo et al., 1999).

Free radicals are structures that contain unpaired electrons in their atomic or molecular structure. Reactive oxygen species and oxygen-free radicals are not stable and could easily react with biomolecule (Shoib and Shohid, 2015). Oxygen is converted into water in the mitochondrial electron transport system. During this process, 2-3% of the oxygen did not into water and causes the formation of oxygen-derived radicals. The oxygen molecule accepts an electron to form a superoxide radical, accepts two electrons to form hydrogen peroxide radical and then forms the

hydroxyl radical (Büyükuslu and Yiğitbaşı, 2015). Radicals could react with other radicals or molecules once they are formed. Free radicals could react with lipids, nucleic acids and proteins leading to damage their structure. Antioxidants are substances that prevent or retard the oxidation of cellular structures. The plants are used as antioxidants due to their carotenoid, flavanoid and phenolic content. Since they do not have any adverse effects, several studies have been conducted on the topic (Okatan et al., 2016; Gülçin 2012; Peschel et al. 2006; Okatan, 2018).

There are only a few studies on *Nepeta viscida* endemic species in the literature. This study was conducted to determine the free radical scavenging effect, total phenolic substance content and total oxidant status, total antioxidant status, and mineral content of the methanol and acetone extracts of the species.

MATERIALS AND METHODS

Plant material

Nepeta viscida plant was collected in Ulupınar village, Banaz/Uşak at an altitude of 1341 m in June and it was identified by Dr. Mustafa Kargıoğlu. The plant sample (35S 0746121, UTM 4276015) was stored at Afyon Kocatepe University Science and Literature Faculty Herbarium.

Preparation of the Plant Extract

Mixture of roots and parts of stem of *Nepeta viscida* plant was used. These parts were divided into small pieces and dried in the shade at ambient temperature. To prepare the extracts, 400 mL of solvent was added to 20 g of pulverized *Nepeta viscida*. The resulting extracts were filtered with filter paper and the solvents were removed by rotary evaporator. Free radical scavenging activity and total phenolic content were determined in these prepared extracts (Gülçin, 2005).Total antioxidant status and total oxidant status were determined after sonicating (10 min)1 g dried and pulverized plants after adding 10 mL solvent. The samples were filtered with filter paper and then centrifuged. The supernatant was removed and centrifuged again to be used in the analyses (Dikilitaş et al. 2011).

To determine the mineral content of *Nepeta viscida*, 0.5 g dried and pulverized plant were placed in a microwave to decompose the organic components (Aksoy and Sözbilir, 2015).

Method

Determination of the total phenolic content

Total phenolic content was determined by Folin-Ciocalteu method. Folin-Ciocalteu reactant was added to plant extracts and standard antioxidant solutions. After Na2CO3 was added, the mixture was stored at ambient temperature for 2 hours. The absorbance of the mixture was measured at 760 nm. The results were calculated with the calibration curve of gallic acid used as standard and reported as gallic acid equivalent (Slinkard and Singleton1977) ($r2 = 0.9843$ Absorbance ($\lambda 760$) = $0.6995x + 0.2842$).

Determination of free radical scavenging activity

The free radical scavenging activities of the methanol and acetone extracts obtained from root and stem parts of *Nepeta viscida* were determined with the Blois method (1958). DPPH. (2,2-diphenyl-1-picryl hydrazine) was used as free radical. Ethanol was added to the samples at concentrations of 45, 90 and 135 µg/mL. Then, stock DPPH solution was added. After incubation for 30 minutes at ambient temperature and in the dark, the absorbance was recorded at 517 nm against the ethanol blind. The extracts that contain antioxidant cause the reduction of the purple color due to DPPH and the absorbance to drop. The reduction in absorbance provides the remaining DPPH solution amount, i.e. the free radical scavenging activity (Blois, 1958).

Total antioxidant/oxidant status and OSI

Total antioxidant status of *Nepeta viscida* was measured using commercial kits (Rel Assay Kit Diagnostics, Türkiye).The kits work based on the principle of ABTS+ oxidation by the reaction of 2,2'-azinobis-3-

ethylbenzothiazoline-6-sulfonic acid (ABTS) with H2O2 in acidic medium and then ABTS radical losing its prior blue and green color. The intensity of the color is based on the antioxidant amount and antioxidant capacity. This absorbance of the color is measured spectrophotometrically at 660 nm (Dikilitaş et al. 2011).

The total oxidant capacity of the extracts was measured with commercial kits (Rel Assay Kit Diagnostics, Türkiye). This method is based on the oxidation of the Fe+2 complex in the plant to +3 valence iron complex by oxidants. The resulting Fe + 3 xylenol form a colored complex with orange. Color intensity varies based on the amount of oxidant in the sample. The absorbance of the color is measured spectrophotometrically at 530 nm (Dikilitaş et al. 2011).

Oxidative stress index (OSI) is an indicator of oxidative stress. It was calculated by dividing the total oxidant status (TOS) by the total antioxidant status (TAS).

Mineral substance

A microwave oven was used to deform the organic compounds in the samples. 0.5 g *Nepeta viscida* sample was placed in Teflon sample containers. Nitric acid, hydrogen peroxide and perchloric acid were added to the sample. The product was placed in the microwave to warm up. The samples were kept at 90-150 ° C in the microwave for a certain period. The samples then were taken out of the oven and stored to reach the ambient temperature and transferred to 10 mL volumetric flasks. The samples were then added 18.2 MΩ cm ultra-pure water to reach 10 mL. The bio-element concentrations (Al, Ba, Bi, Ca, Cr, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb and Zn) in the samples taken from the volumetric flasks were measured (Aksoy and Sözbilir, 2015) by ICP-OES (Spectro Genesis, Germany)

Statistical Analyses

Antioxidant and antiradical analyzes were the average of the three analyzes performed separately. Data analysis (descriptives) was conducted with SPSS (version 15 for Windows, SPSS Inc.) software and the values were recorded as mean ± standard deviation.

RESULTS AND DISCUSSION

Lamiaceae is an important family represented by 45 genera, 565 species and 735 taxa in the Turkish family. (Gedik et al., 2016). The study was conducted to determine the fatty acid content in 5 Nepeta species, including *Nepeta viscida*. In this study, it was found that *Nepeta viscida* seeds contained high total fatty acids compared with the other species (36.2-49.8%). Especially their essential fatty acid content (linolenic acid: 49.8-58.5 and linoleic acid: 10.9-23.5) was remarkable. Furthermore, they contained oleic acid, palmitic acid and stearic acid (Kiliç et al., 2007).

The main phytochemical found in the Nepeta species is nepeta lactone. Nepeta species indigenous to Turkey are classified into two groups based on the nepeta lactones they contain: high rate (60-95%) and low rate (10-46%). *Nepeta viscida* species essential oils, which is considered in the low content group, contain β-pinene, α-terpineol, germacrene and spathunelol. Nepeta species also include di-, tri-, sequiterpenes and iridoids (Kokdil et al., 1999; Takeda et al., 1999).

Phenolic compounds are water-soluble substances found in plants. They are studied in two sections as phenolic acids and flavonoids. Phenolic compounds contain aromatic rings in addition to at least one -OH group. They can perform radical scavenging and metal chelating. Antioxidant activity of their phenolic material is related to the number of hydroxyl groups in their structures and the position of the hydroxyl group. Due to the above mentioned structure, they are molecules with antioxidant properties. The phenolic compounds in the plant protect the cell against oxidative damage induced by reactive oxygen species or free radicals (Etherton et al., 2002; Ghasemzadeh and Ghasemzadeh, 2011).The present study was conducted to determine the total phenolic substance content, free radical scavenging activity and total oxidant status, total antioxidant status and mineral content of the

methanol and acetone extracts of Nepetaviscida. The antioxidant and antiradical methods were used to compare the findings with standard material such as gallic acid and BHT. The total phenolic compound content in the methanol and acetone extracts obtained from root and stem parts of Nepetaviscida species was determined. For this purpose, gallic acid was used as the standard phenolic compound and graphs were plotted. The total phenolic compound content in both extracts was calculated as gallic acid equivalent (GAE) using the formula obtained in the standard graph (r2: 0.9843). The standard gallic acid graph prepared for this purpose is given in Figure 1.

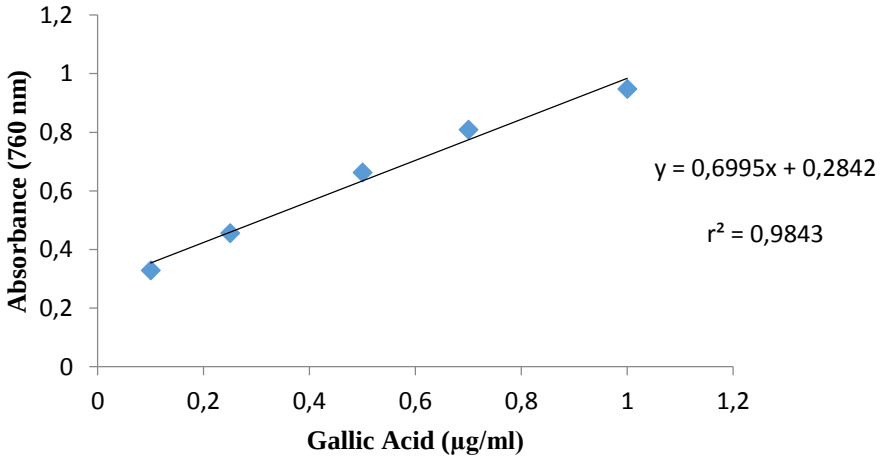

Figure 1 Gallic acid standard curve for total phenolic content

The total phenolic compound content present in 1 mg ethanol and methanol extracts obtained from root and stem parts of *Nepeta viscida* plant is presented in Table 1.

Table 1. The total phenolic compound content present in 1 mg ethanol and methanol extracts of *Nepeta viscida* species

	NVM	NVA
The total phenolic content µG GAE/mg extract	128.86±4.52	134.78±9.3

NVA: *Nepeta viscida* acetone extract; NVM: *Nepeta viscida* methanol extract

As it can be seen in Table 1, 1 mg methanol and acetone extracts of *Nepeta viscida* plant contained 128.86 ± 4.52 and 134.78 ± 9.3 µg GAE phenolic componds, respectively. It was observed that the total phenolic content of *Nepeta viscida* methanol and acetone extracts were similar.

There are several plants with antioxidant properties. Antioxidant properties of plants are measured in vivo using DPPH, DMPD, ABTS, TBARS methods, they could also be measured in experimental animals with several in vitro methods. Although DPPH is not a biological radical, it is a durable nitrogen-based marker that is used as a determinant of antioxidant free radical scavenging activity (Kulkarni et al., 2006) It is frequently used to determine the radical scavenging activity of compounds that contain antioxidants. When the purple colored solution interacts with an antioxidant compound, it is reduced to the yellow colored diphenylpicryl hydrazine. In this method, the

DPPH radical is reduced by the antioxidant substances that contain hydrogen donor groups (Enujiugha et al., 2012). The graphs for the radical scavenging capacities of the plant extracts used in the study are presented in Figures 2 and 3. In the present study, the standard graph was constructed with BHT, a synthetic antioxidant compound.

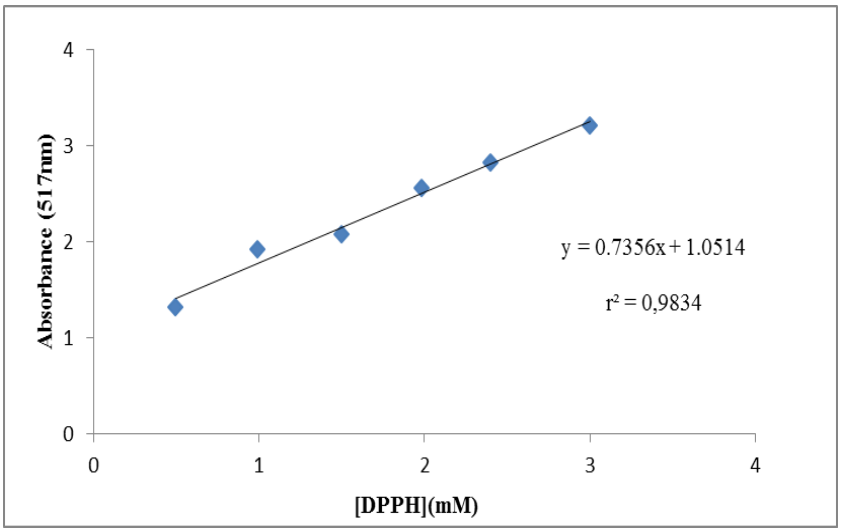

Figure 2. Standard DPPH graph used for determination of DPPH radical scavenging activity

DPPH radical scavenging activities of methanol and acetone extracts of *Nepeta viscida* plant were determined as presented in Figure 2.

Absorbance = 0.7356 [DPPH] + 1.0514

DPPH radical scavenging activities of methanol and acetone extracts of *Nepeta viscida* plant are presented in Figure 3. The figure demonstrates that DPPH radical scavenging activities of the extracts increased in direct proportion to the concentration.

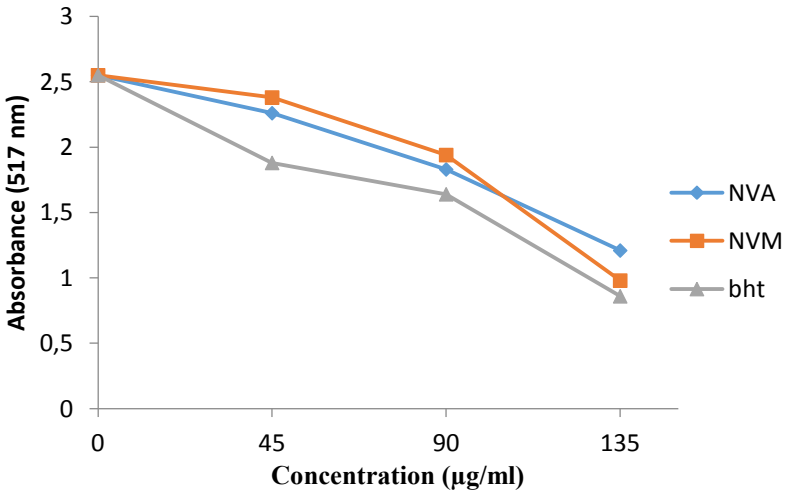

Figure 3. Comparison of DPPH radical scavenging activity in different concentrations (45-135 µg/ml) to BHT

The DPPH radical scavenging activities of the methanol and acetone extracts of *Nepeta viscida* plant and the same activities of the standard antioxidant BHT at 135 µg / ml concentration were determined as BHT~*Nepeta viscida* methanol extract>*Nepeta viscida* acetone extract. DPPH scavenging activities of methanol and acetone extracts of *Nepeta viscida* plant were found to be 66.22% in BHT, 61.6% in methanol extract and 52.5% in acetone extract. The findings demonstrated that especially the *Nepeta viscida* methanol extract exhibited similar results to BHT, a synthetic antioxidant.

Antioxidants reduce the damage induced by oxidative stress by inhibiting the oxidation of oxidant molecules. Antioxidants that have intracellular and extracellular effects are different. While superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) are effective in the intracellular environment, vitamin E and C, albumin, bilirubin, seruloplasmin are significant in extracellular environment (Halliwell, 1991). Total antioxidant status, Total oxidant status, oxidative stress index (TOS/TAS) data observed in methanol and acetone extracts obtained from root and stem parts of *Nepeta viscida* plant are given in Table 2.

Table 2. Total antioxidant status, Total oxidant status, oxidative stress index for *Nepeta viscida*

	NVA	**NVM**
TAS, µmolTroloxEq/g	1.16±0.2	1.24±02.4
TOS, µmol H2O2Eq/g	6.48±2.7	5.53±3.2
OSI, Arbitrary Unit	5.58±0.06	4.46±0.07

NVA: *Nepeta viscida* acetone extract, NVM: *Nepeta viscida* methanol extract, TAS: Total Antioxidant Status, TOS: Total oxidant Status, OSI: Oxidative stress index

The mineral content of the species was determined by ICP-OES. The organic substances in the preprocessed plant were decomposed initially and then the minor and major elements in the structure were determined. The bio element levels in the mixture obtained from root and stem parts of *Nepeta viscida* plant are given in Table 3.

Table 3. Mineral substance levels in *Nepeta viscida*

Mineral substance	**Level (ppm)**	**Mineral substance**	**Level (ppm)**
Al	284.207±9.18	K	1291.82±80.78
Ba	20.096±1.03	Li	0.260±0.02
Bi	1.614±0.31	Mg	989.798±14.72
Ca	1191.7±38.97	Mn	26.651±1.24
Cr	0.671±0.14	Na	226.649±13.45
Cu	3.110±0.27	Ni	0.766±0.12
Fe	232.284±14.56	Pb	1.091±0.16
Ga	0.5135±0.09	Zn	5.957±1.35

In a previous study, anatomical, morphological and ecological properties of *Nepeta viscida* species were evaluated. In this context, soil analysis was conducted and soil pH, salt lime, organic matter, and mineral (P, K, Cu, Zn, and Mn) levels were measured in soil samples (Kiliç et al., 2007). Al, Ba, Bi, Ca, Cr, Cu, Fe, Ga, K, Li, Mg, Mn, Na, Ni, Pb and Zn levels were determined in this study. Bio elements incorporate the structure of antioxidant enzyme systems such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). Certain elements in plant structures participate in several metabolic events by incorporating into enzyme structures. Thus, Fe, Cu, Zn, Mn, Se are the bio-elements that incorporate into the antioxidant enzyme structure in *Nepeta viscida* species, the

elements except Se are present in very high amounts. The species is also significant due to the mineral substances it contains. In addition to the total element content, their chemical forms are very important for bioavailability.

CONCLUSIONS

In conclusion, it could be considered that the phenolic compounds in methanol and acetone extracts of *Nepeta viscida* might have antioxidant effects. It was found that the radical scavenging activity of the methanol extract was close to that of the synthetic antioxidant BHT and higher than the radical scavenging activity of the acetone extract. These TAS, TOS and OSI values were also determined. These values indicated that methanol extract was particularly effective. The minerals in the species were significant minerals (Mn, Zn, Se, Fe, Cu) that incorporate into the antioxidant enzyme structure. Due to the total phenolic content, radical scavenging action, TAS, TOS, OSI values and the mineral substances it contains, it was considered that *Nepeta viscida* could be used in phytotherapy studies, especially in studies on antioxidant action. The present study will contribute to the literature since it is the first study to examine the mineral and total phenolic content, radical scavenging activity, Total Oxidant Status and Total Oxidant Status on the species.

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